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Single-dose desloratadine and montelukast and allergen-induced late airway responses

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ABSTRACT: Montelukast and desloratadine synergistically inhibit the allergen-induced early asthmatic response. Montelukast also suppresses the allergen-induced late asthmatic response, but there are no reports on the effect of desloratadine or the combination on the allergen-induced late asthmatic response.

Atopic asthmatics (n=10) completed a multicentric randomised double-blind crossover study comparing single-dose placebo, 5 mg desloratadine, 10 mg montelukast and the combination administered 2 h prior to allergen inhalation challenge. Methacholine challenges were performed 24 h before and after allergen challenge. Exhaled nitric oxide measurements and sputum inflammatory cell counts were also carried out.

All active treatments significantly decreased the late asthmatic response area under the curve. Combination therapy provided the greatest inhibition compared to desloratadine and montelukast. Montelukast was nonsignificantly better than desloratadine but not as effective as the combination. There was a trend towards a decrease in airway responsiveness following montelukast and combination. Montelukast, but not desloratadine or the combination, decreased exhaled NO levels 24 h after allergen. The allergen-induced increase in sputum eosinophil numbers was significantly suppressed at 7 h with desloratadine and combination therapy, and at 24 h with montelukast and combination therapy.

Single-dose co-administration of desloratadine and montelukast 2 h prior to allergen inhalation clinically abolished the late asthmatic response and eosinophil recruitment.

KEYWORDS: Antihistamine, antileukotriene, asthma, eosinophil, inflammation, sputum

The airway response to inhaled allergen is characterised by airflow obstruction that is usually maximal within 20–30 min of exposure. This is referred to as the early asthmatic response (EAR), which results from immunoglobulin (Ig)-E-mediated mast cell degranulation, release of stored mediators (*e.g.* histamine) and newly synthesised mediators (*e.g.* leukotrienes) that subsequently exert their effects on surrounding tissues, causing bronchoconstriction, plasma exudation and mucus hypersecretion. The late asthmatic response (LAR), which occurs in ~50% of individuals with a positive allergen challenge, is a subsequent episode of airflow obstruction that develops over the 4–8 h after the EAR has spontaneously resolved. The mechanism of the LAR is not fully understood, but immune responses and inflammation play a major role.

Synergistic inhibition of the EAR with the combination of an antihistamine (desloratadine) and a leukotriene receptor antagonist (montelukast) has been documented [1], and we subsequently hypothesised that this combination would also prove beneficial against the LAR, since the LAR is correlated with the EAR and allergen-induced airway inflammation, and since recent data suggest that these agents may have a role in immune and inflammatory responses.

METHODS

Study design

A randomised double-blind four-way crossover placebo-controlled multicentric allergen inhalation challenge investigation was conducted. Assessments were made of exhaled nitric oxide (eNO) levels and airway hyperresponsiveness, and sputum samples were collected at various time-points. All sites used the same standardised methodology for all assessments. The study was registered on ClinicalTrials.gov (NCT00424580). The Saskatoon Health Region Pharmacy Research Unit at the Royal University Hospital (Saskatoon, SK, Canada) provided currently available tablets of desloratadine and montelukast encapsulated with lactose filler in order to produce

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This study is registered at ClinicalTrials.gov (trial number NCT00424580).

STATEMENT OF INTEREST
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identical-looking treatments. Individual treatments were provided to subjects on day 1 of each treatment arm in a small brown sealed envelope. Subjects were instructed to ingest the contents of the envelope 2 h prior to allergen challenge, which was scheduled at ⩾10-day intervals.

Subjects

The subjects either were known dual responders or had undergone screening allergen challenges in order to assess eligibility. Subjects (table 1) were recruited to the study providing the following criteria had been met: baseline forced expiratory volume in 1 s (FEV_1) of ⩾70% of the predicted value; methacholine provocative concentration causing a 20% fall in FEV_1 (PC_{20}) of ⩽16 mg·mL^{-1}; positive skin test to a common aeroallergen; EAR of ⩾20% fall in FEV_1 and LAR of ⩾15% fall in FEV_1; no respiratory infection or change in allergen exposure for 4 weeks prior to enrolment and throughout the investigation; and salbutamol (n=10) had been withheld for⩾6 h prior to testing. One subject was using inhaled corticosteroid and one was using nasal corticosteroid, both on a stable dose prior to and throughout the study. The protocol was approved by the research ethics board of each institution, and all subjects provided written consent prior to the conduct of any study-related procedures.

Allergen inhalation challenge

Serial 2-fold dilutions were prepared from standardised stock allergens (grass, cat and house dust mite (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*)) and diluted in normal saline. Starting concentrations for inhalation were determined by algebraic prediction of allergen PC_{20} using the skin test end-point and methacholine PC_{20} [2]. Allergen challenges began at the same concentration and the same number of concentrations were administered, within a given individual, for each allergen challenge (*i.e.* the same dose of allergen was administered following each treatment). Allergens were aerosolised *via* a Wright nebuliser (Roxon medi-tech, Montreal, PQ, Canada) calibrated to deliver 0.13 mL·min^{-1}. Each concentration was inhaled during 2 min of tidal breathing *via* a mouthpiece and with nose clips in place. Two technically acceptable FEV_1 manoeuvres were performed 60 s apart 10 min after each inhalation was complete. Once the EAR was captured, the response remained untreated and the FEV_1 was assessed at various standardised time-points, up to 7 h after allergen inhalation, in order to capture the LAR [3]. The area under the curve (AUC) for the EAR and LAR were calculated using the trapezoid rule.

Methacholine challenge

Methacholine challenges were performed 24 h before and 24 h after the allergen inhalation challenge using a standardised 2-min tidal breathing method [4, 5]. All methacholine challenges were performed in an identical manner (*i.e.* same starting concentration) within a given subject. The PC_{20} was extrapolated if a concentration of ⩽16 mg·mL^{-1} resulted in a fall in FEV_1 of >17% but <20% [6], and interpolated if the fall was >20% [7]. Methacholine challenges were followed by the administration of 200 μg salbutamol.

Sputum collection and analysis

Sputum was collected 24 h before and 7 and 24 h after allergen challenge. Sputum collection and processing were performed using the methodology of PIZZICHINI *et al.* [8]. In brief, subjects inhaled, *via* mouthpiece, increasing concentrations (3, 4 and 5%, each for 7 min) of hypertonic saline, aerosolised by a high-output ultrasonic nebuliser. Collected specimens were immediately refrigerated and processed within 2 h of collection. Total cell counts were determined using a Neubauer haemocytometer chamber (Hausser Scientific, Horsham, PA, USA) and expressed as the number of cells per millilitre of sputum. Differential cell counts were performed, under blinded conditions, from cytospin preparations stained with Diff Quik (Dade Behring, Newark, DE, USA).

TABLE 1 Patient demographics

Subject no.	Sex	Age yrs	Height cm	Baseline FEV_1 L	Baseline FEV_1 % pred	Allergen	Allergen dilution#	Methacholine PC_{20} mg·mL^{-1}	Medication
1	M	60	168	2.42	74	Grass	1:256	0.56	Salb
2	F	24	155	3.29	106	HDM (Dp)	1:256	2.5	Salb
3	M	30	178	3.41	77	Cat	1:256	0.34	Salb
4	M	25	180	4.25	91	Grass	1:64	1.4	Salb
5	M	24	185	3.49	71	Grass	1:128	1.7	Salb/Bud
6	F	47	168	2.47	84	Cat	1:128	7.0	Salb
7	M	58	180	3.44	90	Cat	1:128	1.3	Salb
8	F	26	165	3.75	110	HDM (Df)	1:64	11.7	Salb
9	M	23	183	5.22	107	Cat	1:32	5.3	Salb
10	F	44	173	3.72	122	Cat	1:4	5.6	Salb/flonase
Mean±SD		36.1±14.8	173.5±9.7	3.55±0.81	93.2±17.3			2.27¶	

Flonase was given at a dose of 2 squirts·nare^{-1}·day^{-1}. FEV_1: forced expiratory volume in 1 s; % pred: % predicted; PC_{20}: provocative concentration of methacholine causing a 20% fall in FEV_1; M: male; F: female; HDM: house dust mite; Dp: *Dermatophagoides pteronyssinus*; Df: *Dermatophagoides farinae*; Salb: salbutamol *p.r.n.*; Bud: budesonide 400 μg·day^{-1}. #: final concentration administered; ¶: geometric mean.

Exhaled nitric oxide

eNO measurements were performed 24 h before and 4, 7 and 24 h after allergen challenge following American Thoracic Society recommendations [9]. Subjects performed an inhalation to total lung capacity *via* a filter/mouthpiece followed by exhalation at a constant flow rate of 50 $mL \cdot s^{-1}$ until the reading was captured. Comparisons were made using the mean of three measurements at each time-point.

Data analysis

Two-way (subject/treatment) ANOVA, followed by pairwise comparison of means (least squared difference) if applicable (Statistix version 7.0; Analytical Software, Tallahassee, FL, USA) was used to examine differences in the end-points under investigation. The study was appropriately (>80%) powered, with 10 subjects, to detect differences in the primary end-point (LAR of 50% inhibition in AUC), and in the secondary end-points (EAR, allergen-induced airway hyperresponsiveness and sputum eosinophil cell counts) [10, 11]. The appropriate sample size for achieving $\geqslant$80% power in detecting a significant change in eNO level is unknown.

RESULTS

All 10 randomised subjects completed the study without incident. Desloratadine, montelukast and the combination all significantly decreased the LAR FEV_1 AUC in a treatment-dependent manner ($p<0.001$ (ANOVA)). Desloratadine reduced the response by 43%, montelukast reduced it by 71% and the combination completely blocked the response (fig. 1). Desloratadine, montelukast and the combination also significantly reduced the mean EAR AUC by 32, 72 and 100%, respectively. The inhibition with combination, however, was not significantly different from that of montelukast alone for the EAR ($p=0.052$). The mean percentage fall in FEV_1 at various time-points following allergen challenge is shown in figure 2. The mean$\pm$SEM doubling dose increase in methacholine PC_{20} was 0.66 ± 0.19 after placebo, 0.82 ± 0.26 after desloratadine, 0.31 ± 0.21 after montelukast and 0.18 ± 0.23 after combination ($p=0.092$ (ANOVA)) (fig. 3a). Sputum eosinophil numbers increased by 23.2% 7 h after allergen inhalation in the untreated arm, whereas the increase following desloratadine was only 10.5% and after combination only 2.8% ($p<0.05$). At 24 h after allergen challenge, the increase in sputum eosinophils was 8.8% following montelukast and 4.5% after combination *versus* 16.2% after placebo ($p<0.05$). The difference between the individual therapies and combination was not significant at either time-point (fig. 4). The allergen-induced increase in eNO concentration was significantly less after montelukast treatment only and only at the 24-h time-point ($p=0.03$) (fig. 3b).

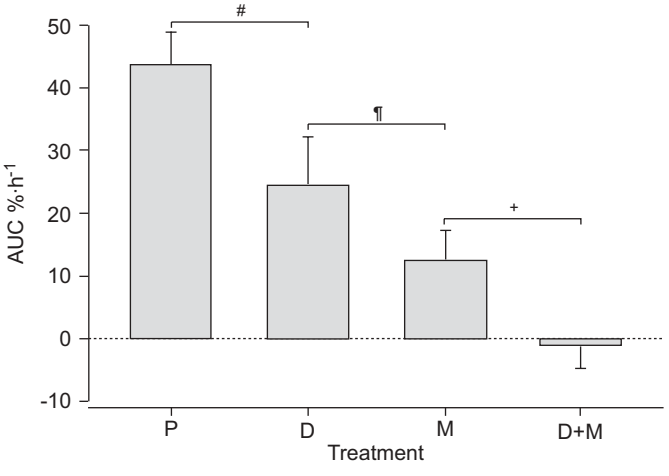

FIGURE 1. Treatment-dependent inhibition of the late asthmatic response (LAR) expressed as mean area under the forced expiratory volume in 1 s curve (AUC) 3–7 h after allergen inhalation (n=10). All treatments significantly decreased the LAR. The difference between desloratadine (D) and montelukast (M) was nonsignificant. P: placebo. #: $p=0.007$; ¶: $p=0.066$; +: $p=0.042$.

DISCUSSION

The present *in vivo* investigation provides new insights into the effects of desloratadine, montelukast and the combination in individuals with mild atopic asthma and a dual asthmatic response. Few clinical studies have investigated the effects of combining a leukotriene antagonist with an antihistamine on the airway response to allergen, and none have looked at a single dose (table 2). Loratadine, the parent compound of desloratadine, has been shown by ROQUET *et al.* [12] to significantly decrease the LAR alone and in combination with zafirlukast following 1 week of high-dose therapy (twice the daily recommended dose of both drugs). The present study has shown that desloratadine, in a single dose, provides the same magnitude of bronchoprotection against the LAR as in this previous study of higher dose and longer duration. Comparison of the leukotriene antagonists suggests that a single dose of montelukast is also more effective than multiple-high-dose zafirlukast. The present study also documents complete inhibition of the LAR with the combination of montelukast and desloratadine, whereas the study of ROQUET *et al.* [12] showed only 75% inhibition of the LAR following the combination of zafirlukast and loratadine. Similarly, a more recent investigation using clinically relevant doses of azelastine and montelukast for 1 week also showed less of an effect compared with the present results [13]. The differences between the present study and these two previous studies may be related to the pharmacokinetic and pharmacodynamic

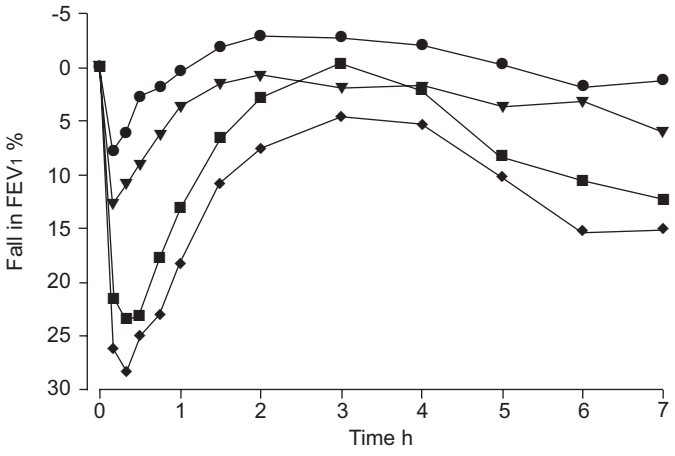

FIGURE 2. Mean fall in forced expiratory volume in 1 s (FEV_1) over the 7 h following allergen inhalation for each treatment arm (■: desloratadine (D); ▼: montelukast (M); ●: D and M; ◆: placebo). $p<0.00001$ (ANOVA; n=10).

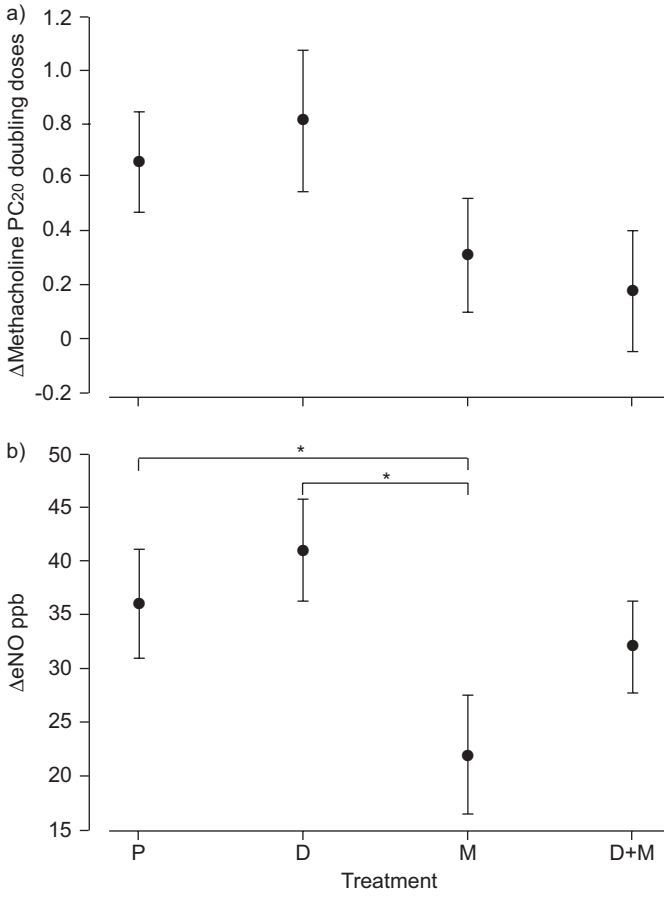

FIGURE 3. a) Airway responsiveness to methacholine expressed as the dose shift (Δ) in provocative concentration causing a 20% fall in forced expiratory volume in 1 s (PC_{20}) from 24 h prior to allergen challenge to 24 h after allergen challenge. Active treatments did not have an effect on the allergen-induced increase in airway responsiveness. A trend is apparent with montelukast (M) and combined desloratadine (D) and M therapy. p=0.092 (ANOVA; n=10). b) Mean absolute change (Δ) in exhaled nitric oxide (eNO) levels 24 h after allergen inhalation. Pretreatment with M resulted in less of an increase than with both placebo (P) and D. *: $p<0.05$; p=0.033 (ANOVA; n=10).

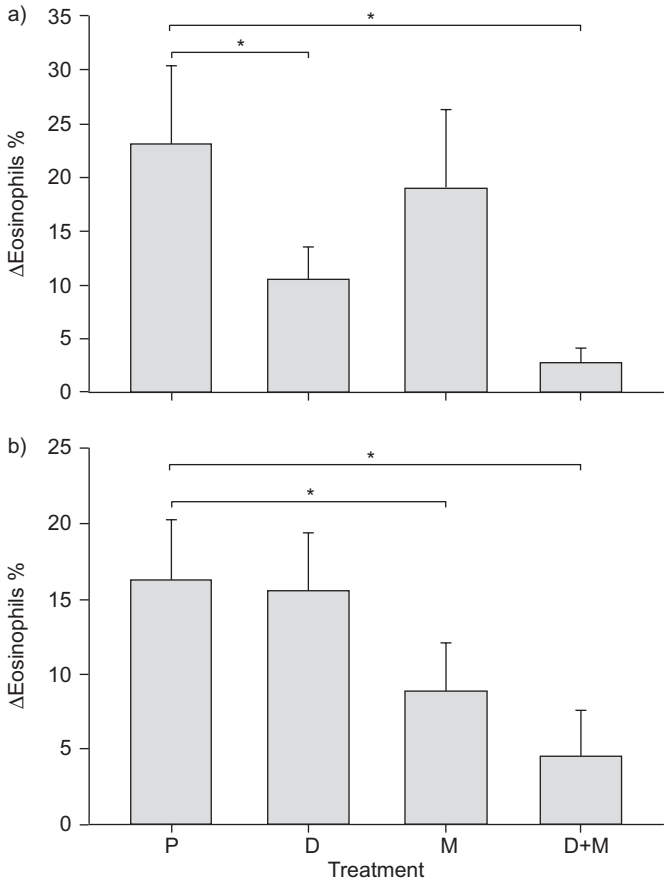

FIGURE 4. Increase (Δ) in sputum eosinophils following allergen inhalation at: a) 7 h; and b) 24 h. a) Pretreatment with desloratadine (D) and combined D and montelukast (M) therapy 2 h before allergen inhalation resulted in a significantly reduced increase in sputum eosinophil numbers at 7 h (p=0.006 (ANOVA; n=10)). b) Pretreatment with M and combination therapy resulted in a significantly reduced increase in sputum eosinophils at 24 h (p=0.036 (ANOVA; n=10)). P: placebo. *: $p<0.05$.

properties of the therapies, allergen challenge methodologies or study design, or may even suggest the development of tachyphylaxis or the onset of tolerance following the longer and higher dosing regimen.

An earlier report documented synergistic inhibition of the EAR with combination therapy that was superior to that of either monotherapy. Montelukast also inhibited the response but desloratadine alone had no effect. Early-response methodology permits the assessment of the response as a doubling-dose shift in allergen PC_{20} [1]. The present study now documents that a single dose of desloratadine significantly inhibits the EAR, assessed as the AUC, as does a single dose of montelukast. The combination completely blocked the response, but did not differ from montelukast monotherapy. If the effects were further assessed as changes in maximal fall in FEV_1 (table 2), the inhibition of the response would be 14% with desloratadine (nonsignificant), 54% with montelukast and 73% with the combination (both significant and no difference between treatments). It should be apparent that the different methods of assessment produce non-equivalent results of equivocal statistical significance, which is most probably the result of the recovery phase of the EAR. Comparison of EAR data and EAR data from an LAR design is therefore difficult.

Direct histamine H_1 receptor and cysteinyl leukotriene receptor 1 antagonism at the level of the airway smooth muscle is an obvious potential mechanism for preventing the bronchoconstriction associated with the LAR by blocking the action of mediators (*i.e.* histamine and leukotrienes) released by recruited inflammatory cells (*e.g.* eosinophils and basophils). However, the single-dose design, pharmacokinetic and pharmacodynamic properties of the drugs and the response to combination therapy render this rationale unlikely as the mechanism responsible for the inhibition of the LAR.

Both histamine [14] and the leukotrienes [15] play a role in leukocyte recruitment, and, indeed, evidence is provided here of a decrease in sputum eosinophil numbers with desloratadine and the combination at 7 h and montelukast and the combination at 24 h. A trend of increased efficacy with combination therapy is apparent, but the difference between

TABLE 2 Dose and effects of histamine H_1 receptor blockers and leukotriene receptor antagonists on the airway response to allergen

Study	Treatment	Daily dose mg	Duration weeks	Inhibition %			
				EAR		LAR	
				AUC	$\Delta FEV_{1,max}$	AUC	$\Delta FEV_{1,max}$
ROQUET [12]	Loratadine	20	1	25	31	40	32
	Zafirlukast	160		62	62	55	36
	Loratadine/zafirlukast	20/160		75	74	74	48
RICHTER [13]	Azelastine	8.0	1	NR	46	NR	43
	Montelukast	10		NR	76	NR	59
	Azelastine/montelukast	8.0/10		NR	89	NR	78
Present study	Desloratadine	5	Single dose 2 h prior	32	14	43	19
	Montelukast	10	to allergen challenge	72	54	71	63
	Desloratadine/montelukast	5/10		100	73	100	88

EAR: early asthmatic response; LAR: late asthmatic response; AUC: area under the forced expiratory volume in 1 s (FEV_1) curve; $\Delta FEV_{1,max}$: maximal decrease in FEV_1; NR: not reported.

the two monotherapies and the combination therapy at the two time-points is not significant. Earlier investigations have shown leukotriene antagonists to significantly reduce eosinophil trafficking to the airway following allergen inhalation. LEIGH *et al.* [16] documented less of an increase in sputum eosinophil numbers following 10 days of 10 mg·day^{-1} montelukast at both 7 and 24 h following allergen challenge, and PARAMESWARAN *et al.* [17] also showed leukotriene inhibition of sputum eosinophil numbers at 7 and 24 h after allergen inhalation following 2 weeks of pranlukast. There is at least one report documenting no change in allergen-induced sputum eosinophilia following montelukast [18]; however, it is difficult to directly compare the present data with those of the study of DIAMANT *et al.* [18] due to the different dosing regimens and study design.

Increased responsiveness to directly acting stimuli (*e.g.* methacholine) is another hallmark of the LAR to allergen. The increase in airway responsiveness following placebo (*i.e.* decrease in methacholine PC_{20}) 24 h after allergen inhalation was unaffected by all active treatments. This is the first report of the effects of desloratadine on allergen-induced changes in methacholine PC_{20}, and the present results are consistent with previous investigations, which have shown no change in airway responsiveness following allergen exposure and pretreatment with a H_1 blocker [19, 20]. The literature surrounding the effect of leukotriene antagonists on allergen-induced changes in methacholine responsiveness is controversial. PALMQVIST *et al.* [21] documented no change in methacholine PC_{20} following 8 days of montelukast monotherapy; conversely, however, 10 mg·day^{-1} montelukast for 10 days decreased the response, as shown by LEIGH *et al.* [16], and 300 mg *b.i.d.* pranlukast for 2 weeks also prevented the allergen-induced increase in airway responsiveness to methacholine, as shown by PARAMSEWARAN *et al.* [22]. TAYLOR and O'SHAUGHNESSY [23] have also shown an inhibitory effect on the allergen-induced increase in airway responsiveness to histamine following single-dose administration of a leukotriene receptor antagonist (ICI204.219; developed as zafirlukast) given 2 h prior to allergen challenge. Although the present investigation is similar to that of TAYLOR and O'SHAUGHNESSY [23] (*i.e.* single dose administered 2 h prior to allergen challenge), direct comparison is difficult due to the choice of directly acting agent (*i.e.* histamine *versus* methacholine) and differences in the times that the measurements were made. Most allergen challenge studies assess this parameter using 24 h before/after allergen challenge data. In the study of TAYLOR and O'SHAUGHNESSY [23], measurements were made ~3 h before and 7 h after allergen challenge.

The relationship between airway inflammation and airway hyperresponsiveness following allergen exposure remains unclear. The present data support a relationship between these parameters in that a reduction in sputum eosinophil numbers parallels a trend towards a significant decrease in airway hyperresponsiveness with montelukast and the combination at the 24-h time-point. Additionally, at this time-point, desloratadine did not affect eosinophil recruitment and there was no change in the increase in airway responsiveness to direct stimuli. Although interpretation would have been difficult (smaller airway calibre compared to the pre-measurement comparator due to the presence of the LAR), it would have been interesting to have measured the methacholine PC_{20} at the 7-h time-point.

With the exception of montelukast, which significantly suppressed the allergen-induced increase in eNO levels at the 24-h measurement, there were no treatment effects on this parameter. The amount of eNO has been shown to increase following allergen exposure [24], and has been reported to correlate with the degree of eosinophilic inflammation [25]. Even though desloratadine reduced eosinophil influx, H_1 antagonism did not affect eNO at 7 h, suggesting that the source of eNO at this time-point might not be eosinophilic in nature. Conversely, a reduction in eosinophil numbers following montelukast is indeed associated with a reduction in eNO

concentration at 24 h. The temporally associated differences are interesting and may be related to the kinetics of eosinophil activation. It may also be worth noting that eNO levels are higher, although not significantly, following antihistamine, and this is perhaps reflected in the observed effect following combination (*i.e.* less than montelukast alone). Relative to bronchial biopsy and bronchial lavage, eNO measurement is an attractive noninvasive procedure for assessing airway inflammation and therapeutic efficacy when incorporated into the allergen challenge model. However, the reported lack of specificity and selectivity may limit the validity and interpretation of the data [26]. It must also be acknowledged that the present study may not be appropriately powered to detect a significant change in eNO concentration.

The biological role of histamine in the pathogenesis of asthma is, once again, an area of great interest. Many of the cells involved in the inflammatory process and the immune response possess histamine and/or leukotriene receptors. In addition to leukocyte recruitment, histamine may have a role in regulating the phenotype of dendritic cells and T-cells [27], direct T-cell trafficking [28] and influence cytokine signalling [29]. Whether or not any of these potential mechanisms can explain the present results needs to be investigated.

It is well documented that histamine and the leukotrienes are indeed important in the manifestation of the EAR. The current investigation provides clinical evidence that these mediators alone, but to a greater extent in combination, are also important in orchestrating the LAR. Current therapeutic options for suppressing the LAR include long-acting β_2-agonists (short-acting if used following the EAR), single-dose inhaled glucocorticosteroids, combination therapies such as Symbicort® and Advair®, and anti-IgE. Importantly, however, there is a subpopulation of individuals with atopic asthma who are treated only with infrequent short-acting bronchodilators. Although the relief provided by the use of rescue bronchodilators is beneficial, prophylactic prevention *versus* masking the response with functional antagonism is perhaps preferred. It is, therefore, these individuals who may benefit from combined desloratadine and montelukast therapy when exposure to a triggering allergen is imminent.

In summary, concurrent, single-dose administration of desloratadine and montelukast blocks the airway response to inhaled allergen for $\geqslant$7 h and suppresses eosinophil influx for up to 24 h. Future investigations regarding potential mechanisms are of significant interest.

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